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Bacteriological Study of Catfish, *Claria gariepinus,* from Fish Pond Sources in Akungba-Akoko Community, Nigeria

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Research Article

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ABSTRACT

Aim: To determine the microbiological quality of catfish meant for public consumption in the university community, Akungba-Akoko.
Study design: Cross-sectional study.
Place and Duration of Study: Department of Microbiology, Adekunle Ajasin University, P.M.B. 01, Akungba-Akoko, Nigeria, between May 2010 and June 2011.
Methodology: Fresh catfish*, Claria gariepinus,* sample obtained from typical fish pond in Akungba-Akoko was subjected to microbiological Investigation in the Laboratory. Nutrient Agar, Eosine Methylene Blue Agar and Man Rogosa Sharpe Agar were generally used for isolation and maintenance of cultures during the study. Moreover a pour plate technique was used for the estimation of the total bacterial and coliform counts.
Results: The total plate count of fish skin samples gave high bacterial count of 65 x 10^2 cfu/ml, the coliforms count was 7.0 x 10^1 cfu/ml, while the anaerobic organisms encountered gave a value of 20 x 10^1 cfu/ml. Similarly, bacterial count of 2.25 x 10^7 cfu/ml coliform count of 1.35 x 10^4 cfu/ml and 6.5 x 10^4 cfu/ml anaerobic organisms were obtained from gills. The isolated bacteria species identified were *Bacillus spp, Staphylococcus spp, Streptococcus spp*, *Micrococcus spp,* and members of enterobacteriaceae which include *Escherichia coli* and *Klebsiella spp* were found in the skin of the fresh fish. Other complex forms of bacterial species were also encountered in the gills of catfish sample used for this study. This includes *S. aureus, E. coli*, *Bacillus spp.* The total aerobic counts of the *Clarias gariepinus* (Catfish) sample were determined and the results of this study shows that the largest numbers of anaerobic microbes were found in gills.
Conclusion: The study suggests adequate monitoring of our fish ponds with a view of adding some antibiotics to their feeds to reduce infectious agents from this source.

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1. INTRODUCTION

Bacteria are widely distributed in nature and it populates most of our food products including Catfish, *Claria gariepinus* which is the most commonly cultivated fish in fish ponds in Akungba-Akoko, hence the need for microbiological study of this food source. The catfishes belong to the family clariidae which is referred to as "Omnivorous scavengers". This categories of fish lack scale and they posses barbels (feelers). They display an auguilliform body; dorsal and fins are extremely long. They also have pectoral fin, head, mouth and respiratory origins. These enable the fish to stay for some time outside water, making use of atmospheric oxygen (Sydenham, 1975). Catfish species live in inland or coastal waters except Antarctica. Catfish have inhabited all continents at one time or the other. Catfish are the most diverse in the tropical South America, Africa and Asia (Lundberg, 2007). More than half of all catfish species live in America. There are only the Ostariophysans that have entered freshwater habitats in Madagascar, Australia and New Guinea (Bruton, 1996).

Catfish is widely cultivated in different parts of the world. According to Durborow (2000), Catfish farming in the United States began in the early 1960's and has grown to 189,000 acres (in January, 2000). Opportunities for raising catfish profitably in Kentucky are present due to a large pay lake market that consumes about 2 million pounds of catfish a year. Kentucky-based live-haulers who deliver fish to the pay lakes would prefer to get fish from their own state if they could find enough reliable producers. Morris (1993), in his study also stated some conditions that may influence the cultures of certain broad species of catfish. According to this investigator, Channel catfish are known for their ability to withstand lower water quality conditions, but limits do exist. These fish require dissolved oxygen of at least 4 parts per million (ppm) or mg/l for routine maintenance, become stressed at 3 ppm and will die at 1-2 ppm. Chronic low levels of ammonia will adversely affect their health and growth.

Catfish is of considerable importance; many of the larger species are farmed and fished for food. Many of the smaller species particularly the genus crydoras are important in the aquarium hobby (Nelson, 2006). Clariid fishes including catfish are commonly referred to as mud fishes because they are restricted to the bottom of the water lying on the mud which forms substantial part of their diet (Teugels et al., 1982). There are some protozoan infections that may affect *Clarias*. Few serious diseases are caused by protozoa. Among the most important protozoa for clarid are the species of *Trichodina* and *Trichodinella* which makes up *Trichodina* complex. They are saucer-shaped and have a short hair-like structure called cilia on outer cuticular ring which causes damage to the surfaces as the parasite attaches and an inner circle of toothed denticles which make these protozoa easily recognizable. Affected fish have a darkened appearance and the skin is often seen to be grey and flaking off. They are inactive and generally do not feed. Affected fish show signs of irritation and may "flash" that is swim on their side for a second or two and rub against the bottom as if to try to scrape parasites off.

Another form of clarid infectious agent is *Gyrodactylus spp.* These monogenean parasites have only been found on the skin but they are often present in significant numbers. They are viviparous or live-bearing and so when conditions on the skin surface are suitable e.g. when there is skin damage, and especially when fish are stressed, they can very rapidly build up in numbers. They are often quite on examination and readily seen under low power

magnification. They are responsible for massive losses when, under as yet unexplained circumstances, a sudden population explosion take place in apparently healthy fish.

Similarly, there is Myxosporidia organism, Cysts of *Henneguya spp.* This is found on skin and gills of catfish in the form of small within nodules. When the cyst is squashed large number of spores can be seen under the microscope. Individual cysts can be seen on fish of all sizes but large numbers generally occur on fig and the results may be serious. Occasionally very heavy infections have been recorded and are thought to cause heavy mortality. Another form is *Edwardsiella ictaluri* (Enteric Septicemia of Cat Fish). They are gram negative motive pleomorphic curved rod, it primarily affects fingering and yearing catfish and the clinical signs of enteric septicemia of catfish closely resemble those of other systemic bacterial infections. Autolysis, oxidation and bacteria activities spoil fish like meat. The fish fresh is autolysed more quickly due to presence of fish enzymes and because of the less acid reaction of fish that favours microbial growth (Sugita et al., 2002). Many of the fish oils seem to be more susceptible to oxidative deterioration than most animal fats. The lower the pH of fish flesh the slower in general bacteria decomposition (Tsukamoto et al., 1990).

In general this study helps to determine the bacteria load present on the skin of the fresh water fish (Cat fish) with relevance to human health after consumption and to isolate the microorganisms that is present on the skin of cat fish. This will also form a benchmark for environmental monitoring of this aquaculture zones.

2. EXPERIMENTAL DETAILS

2.1 Culture Media Used

Culture media used for this study include Nutrient agar, Eosine Methylene blue agar and Man Rogosa and Sharp (MRS). These mediums were sterilized by autoclaving at 121°C for 15 minutes. The inoculating loops and wire were sterilized by flaming in the spirit lamp until red hot, working bench surfaces were decontaminated by the application of disinfectants, antiseptics solution such as dettol antiseptic solution and 70% alcohol.

2.2 Sample Collection

The catfish used for this study, *Claria gariepinus* were obtained from a fish pond in Akungba-Akoko, Ondo State. The fish sample was transported alive in plastic containers (covered with net) to the Microbiology Laboratory of Adekunle Ajasin University, Akungba-Akoko.

2.3 Sample Preparation for Microbiological Study

The fish samples were cleaned with cotton wool and sterile distil water, after which they were cut with a sterile knife. One gram of the skin of the fish were cut and pounded using a pestle and the fish sample was serially diluted. One ml of the appropriate diluents was transferred into sterile Petri dishes and then a pour plate technique was used to enumerate the bacteria counts by adding NA, EMB, MRS into different petri dishes respectively.

The mixture was left to solidify, plates were turned upside down and set inside incubator for 24 hours at 37°C. Observation of colonies began after one day.

2.4 Stock Culture of Pure Bacteria Isolates

These plates were incubated at 37°C for 24 hours and pure isolates obtained were stored on slants of Nutrient Agar in the refrigerator at 4°C. Inoculums from these sources were used for the study as desired.

2.5 Bacterial Characterization and Identification

Bacterial colonies were observed after 18-24 hours of incubation for their colonial characteristics such as shape, colour, size, edge elevation, transparency and surface texture. Similarly, the isolates were Gram stained to differentiate the organisms into Gram negative and Gram positive by microscopic examination of stained preparation. Hanging drop preparations of the isolates were made on cavity slides and examined microscopically for motility. A good number of coliform isolates were motile. Other biochemical reactions including catalase, indole, starch hydrolysis and sugar fermentation were intensified. One percent of sugars such as glucose, sucrose, lactose, maltose and others were used in a basal fermentative medium to determine the ability of the organisms to utilize the appropriate carbon sources signified by acid production or the change in colour of the medium and production of gas in Durham tube provided for the test.

3. RESULTS AND DISCUSSION

This study helps to determine the microbial load of fish and the bacteria species present on the skin of the fresh water fish, cat fish (Table 1a). The total plate count of fish sample which have the highest microbial count of 65 x 10^2 cfu/ml on Nutrient Agar (NA) and 7.0 X 10^1 cfu/ml coliforms on Eosine Methylene Blue (EMB) Agar and 20 x 10^1cfu/ml on Man Rogosa Sharpe (MRS) Agar for the anaerobic organisms are shown in Table 1b. Similarly, bacterial count of 2.25 x 10^7 cfu/ml coliform count of 1.35 x 10^4 cfu/ml and 6.5 x 10^4 cfu/ml were obtained from gills (Table 2). The cultural characteristics of isolates on solid media used including the biochemical characteristics and identification of bacteria isolates are shown in Table 3.

Different species of bacteria obtained from each of the plates using the solid medium include, *Bacillus spp, Streptococcus spp, Klebsiella spp,* and *Pseudomonas spp.*

Table 1a. Scientific classification of catfish

Kingdom	*Animalia*
Phylum	*Chordata*
Super class	*Osteichthyes*
Class	*Actinoptergii*
Super order	*Ostariophysi*
Order	*Siluriformes*

Table 1b. Total bacteria count of skin of cat fish using nutrient agar, EMB and MRS

Sample	Total Bacteria Count NA (cfu/ml 10^2)	Total Coliform Count EMB (cfu/ml 10^1)	Total Anaerobic count MRS (cfu/ml 10^1)
(1)	65	7	20
	cfu/ml 10^4	cfu/ml 10^2	cfu/ml 10^2
(2)	20	3	10

Table 2. Bacterial count of gills of cat fish using nutrient agar, EMB and MRS

Sample	Total bacterial count (cfu/g)	Coliform count (cfu/g)	Anaerobic count (cfu/g)
C. gariepinus	2.25×10^7	1.35×10^4	6.5×10^4

This study shows high microbial load of 65 x cfu/ml 10^2 from cat fish sources sampled. Similarly, some coliforms, 7 x cfu/ml 10^1 and anaerobic organisms populating 20 x cfu/ml 10^1 were also obtained (Table 1 b). In the gills, bacterial count of 2.25×10^7 cfu/ml, coliform count of 1.35×10^4 cfu/ml and 6.5×10^4 cfu/ml were recorded (Table 2).

This high bacterial population may be due to the discharge of waste material into water bodies upon which the fish species feed or it might result from flooding during rainy season.

Many investigators (Sugita et al., 1997; Shewan, 2000; Shewan and Hobbs, 1990; Okaeme, 2006) have isolated different species of bacteria from the skin of the fresh water fish (catfish) including *Bacillus species* from the skin of sea water fish. Tsukamoto et al. (1990) isolated *Proteus* species from some fresh water. H. Sugita reported that *Staphylococcus spp, Escherichia coli* were isolated frequently from the skin of fresh water fish. He concluded that the skin of fresh water fish were the natural habitat of these bacteria. Some investigations reported that the skin of the *Claria species* contained *Klebsiella spp, Pseudomonas spp*, *Micrococcus spp* as the predominant genera.

Some researchers concluded that predominant genera are *Pseudomonas, Staphylococcus* and the member of the family Enterobacteriaceae in the skin of fresh water fishes. Various bacterial species were encountered in the gills of catfish sample used for this study. This includes *S. aureus, E. coli, Bacillus spp* and others. The total aerobic counts of the Catfish sample are shown in table 3b. Based on the results of this study the largest number of microorganisms in gills of *Clarias gariepinus* was found in anaerobic count.

Table 3a. Cultural characteristics and biochemical characteristics of isolated bacteria from skin of cat fish

Code		Gram Staining	Shape	Glucose	Maltose	Lactose	Mannitol	Sucrose	Motility	Ornithine	Indole	Catalase	Probable organism
ATKNI	Rhizoid, Raised edge Undulate, Transparent Milky	+	Rod	A-	A-	- -	A-	-G	+	+	+	+S	*Bacillus spp*
ATKN2	Irregular, Low Convex Entire, Transparent milky	+	Cocci in chain	AG	- -	AG	- -	AG	-	+	-	-	*Streptoccus spp*
ATKN3	Circular , Flat or effuse Lobate, Opaque, Creamy	+	Cocci in cluster	A-	A-	A-	A-	A-	-	+	-	+	*Staphylococcus spp*
ATKN4	Circular, Low convex Tentate, Opaque, Creamy	-	Rod	A-	-G	A-	AG	AG	+	+	+	+	*Proteus spp*
BTKE1	Rhizoid, Flat or effuse Tentate, Transparent, Milky	-	Rod	AG	A-	AG	AG	AG	-	+	-	+	*E. coli*
BTKE2	Filamentous, Raised edge, Undulate, Transparent, Milky	-	Rod	A-	- -	- -	- -	- -	+	-	+	+	*Pseudomonas spp*
BTKE3	Irregular, Flat or effuse Entire, Transparent, Milky	-	Rod	-G	-G	AG	AG	AG	-	-	-	+	*Klebsiella spp*
BTKE4	Circular, Flat or effuse Fimbrate, Opaque, Milky	+	Cocci	AG	AG	A	A	A	+	-	-	+	*Micrococcus spp*
CTKMI	Filamentous, Flat or effuse Tentate, Opaque, Milky	-	Rod	AG	A-	AG	A-	AG	+	+	+	+	*Proteus spp.*
CTKM2	Rhizoid, Low convex Tentate, Transparent, Creamy	-	Cocci	AG	- -	AG	- -	AG	-	+	-	-	*Micrococcus spp*
CTKM3	Irregular, Raised edge, Tentate Transparent, Milky	+	Cocci in chain	AG	- -	AG	- -	AG	-	+	-	-	*Streptoccus spp*
CTKM4	Circular, Low convex, Crenated Opaque, Milky	+	Cocci in cluster	A-	A-	A-	A-	A-	-	+	-	+	*Staphylococcus spp*

KEY: A= Acid Production; AG= Acid and Gas Production; G= Gas Production; - - = No gas and Acid Production

Table 3b. Cultural and biochemical characteristics of microorganisms isolated from gills of catfish

Sample code	Media used	Cultural characteristics of isolates	Gram Reaction	Catalase	Coagulase	Mannitol	Fructose	Galactose	Glucose	Lactose	Shape	Probable bacterium
CF 1	Nutrient Agar	Irregular, Raised, Rhizoid Smooth, Translucent White & Milky	-	+	+	AG	A G	A G	A G	-	Cocci in cluster	*Staphylococcus aureus*
CF 2	Nutrient Agar	Circular, Flat, Lobate Rough, Transparent Whitish	+	+	+	AG	-	A	A G	A G	Short rod	*Escherichia coli*
CF 3	Nutrient Agar	Circular, Raised Lobate, Smooth Transparent, Whitish	-	+	+	AG	-	-	A G	A G	Small rod	*Enterobacter aerogenes*
CF 4	Nutrient Agar	Irregular, Convex Entire, Rough Transparent, Whitish	+	-	-	AG	A G	A	A	A G	Cocci in chain	*Streptococcus spp*
CF 5	MRS	Circular, Convex Lobate, Smooth Opaque, Milky	+	-	-	AG	A G	A G	A G	-	Rods	*Lactobacillus spp*
CF 6	MRS	Circular, Raised, Lobate, Rough, Opaque, Milky	+	-	+	AG	A G	-	A G	-	Small rod	*Clostridium spp*
CF 7	MRS	Circular, Flat, Rhizoid Rough, Transparent Whitish	+	-	-	AG	-	-	A G	-	Rods	*Lactobacillus spp*
CF 8	MRS	Irregular, Convex Entire, Rough Opaque, Milky	-	-	-	AG	-	-	A G	A	Short rod	*Escherichia coli*
CF 9	EMB	Irregular, Raised Entire, Rough Transparent, Whitish	+	+	-	AG	A G	-	A G	-	Cocci	*Enterococcus faecalis*
CF 10	EMB	Circular, Raised Lobate, Smooth, Opaque Milky & Translucent	+	+	+	AG	A G	A	-	A G	Small rod	*Pseudomonas alcaligenes*
CF 11	EMB	Irregular, Convex Lobate, Smooth Translucent, Whitish	-	-	+	-	A G	-	A G	A G	Small rod	*Klebsiella pneumoniae*
CF 12	EMB	Circular, Convex Lobate, Rough Transparent, Milky	-	+	-	AG	A	A G	A G	A G	Long rod	*Enterobacter aerogenes*

KEY: A= Acid Production; AG= Acid and Gas Production; G= Gas Production; - - = No gas and Acid Production.

4. CONCLUSION

It was concluded from this investigation that the skin of fish favours microbial growth. It is prone to microbial contacts in water, the common environment for micro-organisms and fish. The skin part of fish was vulnerable to bacteria and this is because the skin of the fish is usually in direct contact with water. High percentage of losses in fish production enterprise is due to microbial infection. Hence, there is need for good fish culture and water management to reduce the occurrence of disease in fish in order to achieve the financial and nutritional benefits of the fish production venture. Water environment for fish must be of good quality and feed for the fish must not be contaminated.

In the context of this study, farmers should be educated on how to observe clinical signs of fish disease and stress, and in this way, temporary treatment could be given before consulting a fish production or fish medicine expert. For general preventive measure, broad spectrum antibiotics could be added to the fish feed and mixed in water at recommended doses (in water bath and when regularly changing the water), this reduces bacterial load, enhance productivity and guide against spread of diseases

Results obtained in this study will also form a benchmark for environmental monitoring of this aquaculture zones and hence improve the productivity of catfish, *Clarias gariepinus* as a proteinous source of food in this university community in Nigeria.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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